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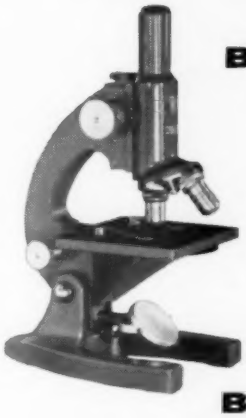

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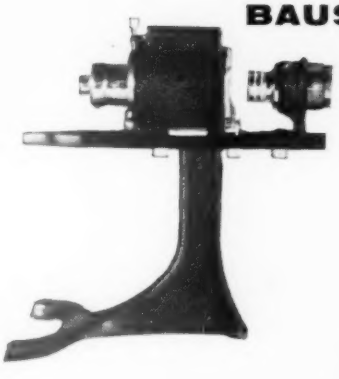

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COVER PHOTO

An electron micrograph (X 10,000) taken of a corn root tip cell wall. The threads are cellulose fibrils, and the oval areas are primary pit fields. Mr. Jerry J. Nisbet, Department of Science, Ball State Teachers College, and Dr. A. T. Guard, Department of Biological Sciences, Purdue University, took the photograph.

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Antidote for Formalin

ELBERT C. COLE
Williams College,
Williamstown, Massachusetts

This article is based upon a portion of an address entitled "Materials and Equipment for the College Course in Biology, and the Role of the Laboratory," which was presented at the Biology Teachers' Conference held at Wabash College on June 24-26, 1954. The conference was sponsored by the Fund For The Advancement of Education as part of the program of The School and College Study For Admission With Advanced Standing. Dr. Cole is the Samuel Fessenden Clarke Professor of Biology at Williams College, Williamstown, Mass.

Under the pressure of many duties and responsibilities, the busy biology teacher is often tempted to order preserved materials from a supply house, rather than to secure and care for living things. The temptation should probably be resisted. In too many courses the laboratory appears as a well-stocked morgue. The perfume of formalin is in the air! It is a sad commentary that such courses, supposedly dealing with "Life Science," are actually dedicated to the dead. To be sure, formalin has its uses, but most teachers well know that living plants and animals in the laboratory provide *an antidote for formalin* and greatly increase student interest in biology.

The main thesis of this paper is that more living material should be used in the biological laboratory. Even when the main work is devoted to dissection, it should be lightened by one or more demonstrations or experiments involving living things. For example, a laboratory period devoted chiefly to the dissection and tracing of the circulatory system in preserved specimens should be highlighted by a demonstration of the circulating blood in a living animal. Of course this procedure is standard practice in a great many courses. Nevertheless, the importance of using living materials can scarcely be over emphasized.

The invertebrates offer abundant opportunity for the use of living material. The following suggestions, with one exception, relate to invertebrate animals. The demonstrations and experiments selected are easy to prepare, require no special apparatus, and are reasonably sure to produce satisfactory results. Some of the methods can be documented. Others have reached the author from forgotten sources, via the "grapevine," doubtless much modified in transit.

Protozoa

The Protozoa provide an enormous number of opportunities for demonstration and experiment, as is attested by the voluminous literature on the subject. The few suggestions presented below are selected on the basis of availability of material and ease of preparation. *1. Mixed Cultures.* Small masses of pond scum and organic debris from a ditch, pool, pond, or stream should be placed in finger bowls of water, preferably from the same source. Protozoans, crustaceans, and annelid worms will be noted, usually in small numbers. As decay proceeds, these dishes become teeming cultures, and certain forms become dominant. As conditions change, dominant forms decline and are replaced by others. These cultures may be used for a study of population sequences, and of pH changes as the solutions age. They may be used as stocks for the subculturing of selected species. A medium suitable for subculturing any one of several species may be prepared by boiling four or five wheat kernels in 75 ml. of pond water and allowing the solution to cool. The culture fluid should then be seeded with the desired organism. If a culture is exposed to sunlight it will commonly turn green, as a result of the rapid population increase of algae or any euglenoid forms that may have been present in the seeding fluid. If this is not desired, it is advisable to keep the culture in subdued light.

2. *Cultures of Vorticella.* It has been observed that, under normal pond conditions, such protozoans as *Vorticella, Carchesium,* and *Epistylis* are commonly attached to the body surfaces of tadpoles and other aquatic animals. Cultures may be made by placing crayfish or large tadpoles in finger bowls of pond water. Within a day or two, a thin brown scum appears on the surface of the water, and remains for a few days. Study of this scum often reveals the presence of *Vorticella* or other peritrichs. The quick coiling and slower uncoiling of the contractile stalk of *Vorticella* may be readily seen. From descriptions given by Leeuwenhoek, it has been inferred that this animal was the first protozoan that he saw.

3. *Cultures of Paramecium.* Ordinary hay or wheat infusions are useful for rearing *Paramecium* in moderate numbers. For dense cultures of this protozoan, bean-seed infusions may be used (Cole 1935). Four-inch finger bowls containing from 50 to 100 ml. of water are made ready. To each is added one dry bean which has been cut transversely to expose the interior of the cotyledons. The new cultures should be seeded immediately, using about two ml. of hay infusion. The dishes are then stacked and a cover applied to reduce evaporation. An occasional culture will turn sour and the animals will be destroyed. In the others a thick, ropy, gelatinous, bacterial mass develops, and the number of Paramecia increases enormously. The mass should be broken if it grows completely across the surface of the culture. Along the edges of the bacterial masses, the Paramecia become so dense that they appear as cream colored borders. When a drop from such a culture is placed upon a slide, the animals move sluggishly and may be readily studied under high power. A few drops placed in a depression slide and allowed to concentrate through evaporation give a spectacular picture when projected upon a screen.

4. *Quieting Paramecium.* For prolonged study of the structure and many of the activities of *Paramecium,* it is essential that the animal be kept relatively quiet. Many ways have been suggested. Most anesthetics distort or destroy the animal; mechanical methods of retarding movement are usually more satisfactory. The gelatinous nature of the bean-seed infusion, mentioned previously, is a factor which makes the animals move slowly. A one per cent suspension of methyl cellulose in water gives similar results. A drop of this fluid mixed by use of a needle point with a drop of the culture in no way harms the animals. Minute masses of absorbent cotton or of frayed lens paper provide small areas in which Paramecia will be trapped. In any one of these methods, numerous air bubbles may be introduced to limit even further the movements of *Paramecium.* This is accomplished by holding the cover glass about an inch above the preparation and allowing it to drop into place.

The depth of the preparation is another important factor for success in studying the details of structure and activity in *Paramecium.* A "high" preparation allows too much vertical movement of the animal. A "low" preparation is preferable, in spite of the fact that it may dry out sooner. Drying is reduced by using a syringe to make a ring of petroleum jelly on a slide and placing the culture within the ring (Buck 1943). The coverglass is then applied, and gently pressed into position with the point of a needle; a high, medium or low preparation may thus be secured.

5. *Protoplasmic Movements.* The cyclic movements of the food vacuoles in *Paramecium,* and the streaming movements of protoplasm in *Ameba* may be clearly shown in a low preparation by the use of darkfield methods. The mirror of the microscope is moved to one side so that only lateral light enters the preparation, and the background appears dark. Or, the mirror may be deflected so that no light enters the preparation, and sidelighting secured by use of a spot light. Under such conditions, the granules in the protoplasm appear as bright spots, and their movements may be readily followed.

6. *Ingestion in Paramecium.* An ingenious method for studying ingestion has been devised by Buck (1943). Since the amounts he gives provide a larger volume of reagent than would be used by small classes, his directions for preparation have been somewhat modified here. A piece of fresh yeast the size of a pea is boiled in 10 ml. of water to which a trace of congo red dye has been added. Excess dye should be avoided. The cooked yeast should be pink, not red. A syringe is used to make a ring of petroleum jelly on a slide, and a drop

of *paramecium* culture is placed within the ring. Stained yeast is introduced by needle point into the culture. A coverglass is gently pressed down upon the preparation. In spite of the fact that the yeast plants are much larger than the usual food, the Paramecia ingest them readily, and numerous food vacuoles gorged with yeast are formed. Because of the acid condition of the food vacuoles, their contents gradually turn from pink to purple, and at pH 3 become deep blue. The animals become sluggish, gather near air bubbles and bacterial masses, and may there be studied under high power. Movements of the cilia, operation of the contractile vacuoles, and protoplasmic movements may be easily seen.

Acid fuchsin is another dye useful for the study of the feeding reactions of *Paramecium* (Cole, 1934a). A double drop method is used. The slide is ringed with petroleum jelly, and a drop of culture fluid and a drop of one per cent acid fuchsin are placed side by side within the ring. The coverglass is applied and gently pressed into the petroleum jelly until a low preparation is secured. The pellicle of *Paramecium* is impermeable to the dye, but the dye is absorbed on the pellicle in a thin, perhaps monomolecular, layer which gives the animal a pale luminous green color. The surrounding fluid is pink and bacteria are stained a deep red. Under high power one can observe the pink water being swept down the gullet, the red bacteria caught in the swirling currents in the food vacuole, the enlargement of the food vacuole, and finally, the pinching of the tip of the gullet which frees the food vacuole and permits the formation of a new one.

7. *Rate of Vacuole Formation.* If the pellicle of *Paramecium* is permeable to a dye, the staining effects are quite different from those resulting from the use of an impermeable dye. For example, when neutral red 1:5000 is added to a slide of Paramecia, all food vacuoles are stained—red, orange, or yellow, depending upon their pH. In the case of acid fuchsin, the gullet of *Paramecium* is the only avenue by which the stain can enter the interior of the animal. The dye is only slightly toxic. Consequently, a drop of one per cent stain may be added to a drop of culture on a slide, with no apparent ill effects on the protozoans. If such a preparation is made, covered, and then quickly examined under the microscope, it will be noted that only two or three food vacuoles appear red. These are the ones which were formed during the brief time elapsing between the application of the dye and the examination under the microscope. It is possible to time the formation of food vacuoles, and determine the rate of their production.

8. *Extrusion of Trichocysts.* Trichocysts, when extruded, are much longer and thicker than is generally appreciated. The act of extrusion is a spectacular sight and may be easily observed. The double drop method is used. A drop of 1:2000 methylene blue solution and a drop of rich culture are placed side by side, but not touching, on a slide. When the coverglass is applied, the circle appears half blue and half white. The microscope should be focused on the boundary line. Here and there animals will be observed to enter the blue zone. In favorable cases, the trichocysts of the anterior portion, or even of the whole body, will be extruded and instantly stained dark blue. Sometimes the animal retains enough vitality to withdraw, leaving a heap of darkly stained trichocysts resembling a pile of jackstraws. The process may be retarded in individual cases, and one may observe individual trichocysts "explode," one after another. The analogy between this and the popping of popcorn is suggested.

The extrusion of trichocysts, as seen under the compound microscope, suggests that the phenomenon is analogous to the squeezing of toothpaste from a tube. But studies of the trichoycst under the electron microscope have shown that it is a minute preformed structure (Jakus, Hall, and Schmitt 1942; Jakus 1945; Jakus and Hall 1946). Under suitable stimulus it elongates somewhat after the manner of a jack-in-the-box.

9. *Demonstration of the Macronucleus.* Many dyes, including methylene blue, will stain the nucleus of cells. But methyl green is said to be specific for chromatin. One gram of dry stain is dissolved in 500 ml. of one per cent acetic acid. The macronucleus of *Paramecium* stains bright green. The cytoplasm often appears gray or lilac, presumably due to traces of methyl violet (Conn 1953). Methyl green will also cause extrusion of trichocysts; the effect is instantaneous.

10. Reactions of Paramecium. The reactions of *Paramecium* to various stimuli have been extensively studied. The classic work of Jennings (1906) and Mast (1911) laid the foundations for many of these studies. Calkins and Summers (1941) and Wichterman (1953) deal extensively with protozoan structure and behavior.

Many reagents can be introduced under the coverglass of a preparation by use of a pipette with the glass tip drawn out into a very small tube. When 0.5 per cent NaCl is introduced into the middle of a preparation on the slide in this manner, the avoiding reaction of *Paramecium* causes the salt solution to remain free of animals for some time. If 0.02 per cent acetic acid is used, the animals show a positive response and are soon thickly gathered in the acidic area. Thermotropism may be shown by placing two metal blocks, one chilled in the refrigerator, and other warmed to about 30 degrees C., on the microscope stage and placing a slide upon them so that one end is chilled and the other warmed. Several drops of culture are then flooded over the slide. Within a few minutes, the Paramecia will be concentrated in a zone nearest the optimum temperature (26 degrees C.).

The reaction of *Paramecium* to slowly diffusing dyes and other chemicals and to the passage of an electric current may be demonstrated by the use of comparatively simple apparatus. A useful material for making such equipment is methyl methacylate polymer, such as Lucite (Cole 1938). It may be conveniently cut to the size of a microscope slide. Minute pits, made with a hot needle, may be used to hold Paramecia within narrow limits of movement. A long trough gouged or ground out of a piece of this plastic makes it easy to study the free movements of *Paramecium*. Two depressions ground in a piece of plastic and connected by a minute shallow groove provide a device for the study of the effect of diffusing chemicals. One depression is filled with culture, the other with the reagent, and the two connected by laying a thread, to serve as a wick, in the groove between them. By using chloroform as a solvent, edges may be built upon a flat piece of plastic, thus forming a shallow box suitable for studying the response of *Paramecium* to weak electric currents.

Coelenterates

The suggestions relative to this phylum are restricted to *Hydra*, since the use of marine forms is feasible in a limited area only.

11. Feeding. This is a widely used demonstration, and is almost always successful. *Hydra* feeds freely on the crustacean, *Daphnia*. The size of the latter determines to some extent the results of the experiment. Large specimens will often shred *Hydra* by rapid movements of the appendages. Medium-sized crustaceans are readily ingested. Fill the cavity of a depression slide with water, add a single *Hydra* and one or more *Daphnia*. Observe under low power. As soon as the tentacles make contact, the crustacean struggles violently. Sooner or later the prey is drawn toward the mouth and engulfed. The heart beat and movements of the appendages of *Daphnia* continue for some time after the animal has been forced into the gastrovascular cavity. Some time later the indigestible residue is regurgitated.

12. Regeneration Hydra. May be cut in many ways to demonstrate regeneration. One of the simpler ways is to cut off one or more tentacles. For this purpose the edge of a round coverglass is rolled over the base of the tentacle. Regeneration requires several days, and during that time *Hydra* should be kept in cool, clear water.

13. Discharge of Nematocysts. The actual discharge of nematocysts may be observed by placing *Hydra* on a slide in a drop of water, adding a coverglass, and allowing methylene blue 1:2000 to diffuse until contact with the animal is made.

14. Structure of Nematocysts. A specimen of *Hydra* should be placed on a slide, a drop of methylene blue 1:2000 added, and a coverglass applied. Both discharged and undischarged nematocysts will be observed. Undischarged penetrants and solvents often stain so as to show the coiled thread *in situ*. Both types of glutinants, however, usually stain solid blue, showing little of the internal structure.

Flatworms

Several types of flatworms are usually available, and provide material for interesting demonstrations.

15. Phototropism in Planaria. The most widely used flatworm *is Planaria (Euplanaria)*. *Euplanaria maculata* and *E. agilis* show marked

negative phototropism. The under slide of a petri dish should be covered with paper, one half black and the other half white. About one fourth of an inch of pond water is placed in the dish, and 10 planarians added. The dish should be brightly illuminated by a goose-neck lamp. Fifteen or twenty counts should be made, at one minute intervals, to determine how many animals are on the white surface and how many are on the black area. The two sets of numbers should be totaled. Usually, but not always, the black background is favored. Failure of the animals to show consistent negative phototropism may be due to the fact that the black background absorbs and radiates heat to a greater extent than does the white background. Since planarians seeks cooler water, it may happen that their thermotropic response counteracts or neutralizes their phototropic response. If a glass dish filled with water is placed between the light source and the dish containing the animals, heat will be largely absorbed, and a more consistent negative response to light will occur. Response to light will also be clearly shown if planarians are placed in a dish of cool pond water containing several small flat stones.

16. Regeneration in Planaria. Regeneration experiments are feasible if care is taken to keep the dish clean and the water clear and cool. *E. agilis* is excellent for this purpose. The animals are vigorous, the color is black, and regenerated tissue appears white at first, then slowly darkens. This is due to the fact that pigment cells in the old tissue migrate slowly into the newly formed areas. Simple cuts are preferable, such as removal of the posterior third or half of the animal.

17. Feeding in Planaria. Feeding experiments with planarians often fail, possibly due to their extreme sensitivity to slight changes in the composition of the fluid surrounding them. It is, therefore, very important to use scrupulously clean slides and pipettes. A specimen should be placed on a depression slide in a few drops of water from the culture. Minute amounts of food, such as cooked egg yolk, cooked liver, or fresh earthworm tissue, should be added. In favorable cases the proboscis is extended, and the passage of food into it may be observed under low power.

18. Parasitic Flatworms. Flukes may be found in several places in the frog's body. The lungs are the site of *Pneunoneces ranarum* and related species. Remove the lungs from a freshly killed frog and tease the tissues with needles in 0.6 per cent NaCl. If parasites are present they will be seen clearly. The broad, mottled black and white worms are lung flukes. When mounted on a slide and covered, the movements of the digestive tract and the spawning of eggs may be observed.

Yellowish cysts are commonly present on the stomach, intestine and liver of the frog. These contain immature flukes, which may be freed by teasing.

The urinary bladder of the frog often yields several species of flukes. Several of them are conspicuous because of their large suckers.

Tapeworms are present in many fishes and mammals. Perhaps the surest source is an alley cat, but the complications that may arise from an attempt to secure one may make the venture seem scarcely worthwhile! Rats are also a good source. When the intestine of the freshly killed animal is slit open, the tape worms may be lifted with a bent needle and gentle tension exerted until the scolex tears free from the intestinal wall.

Roundworms

There is a vast number of species of roundworms, although that fact is often obscured by reliance upon large ascarid worms for demonstration and dissection. Numerous smaller forms are available and may be used to demonstrate the peculiar lashing movements characteristic of this group.

19. Free-living Roundworms. Free-living forms may be found in vinegar, decaying fruit and vegetables, rich soil, the bark of trees, and many other places. The vinegar "eel," *Turbatrix aceti* is often present in unsterilized vinegar. Fresh apple juice or fresh vinegar may be inoculated; such a culture will thrive for many months, even in a sealed jar or bottle, provided that a generous air space is left. Decaying apples, and turnips with a "soft" spot usually house a teeming roundworm population. If bark from maples, elms, or oaks is placed in a little water, a good colony of roundworms usually develops. Most free-living forms are relatively small, and little internal detail can be made out, even with the use of dyes. Most dyes, unless dissolved in

alcohol, fail to penetrate the cuticle. Alcohol penetrates the cuticle, but greatly distorts the internal organs.

20. Parasitic Roundworms. Parasitic forms are also widespread. Slender *Rhabdias ranae* is usually present in the lungs of frogs. The thicker-bodied *Rhabditis pellio* is present in the nephridia of the earthworm. Under pressure and with reduced lighting, some details of the internal structure may be studied. (For an easy way to remove nephridia from the earthworm, see section 26 of this paper).

Annelids

Oligochaetes are widespread and easily obtainable from water and soil. The examples given belong to this group. The leech has been omitted, although it does lend itself to a number of interesting demonstrations.

21. Small Oligochaetes. Pond cultures commonly yield specimens of *Nais, Chaetogaster*, and *Aelosoma.* In all of these worms, the peristaltic movements of the digestive tract can be easily observed. In *Aelosoma* particularly, the attachment of the protractor muscles to the bundles of setae, and the change in position of the setae as these muscles contract can be demonstrated under low power. *Aelosoma* reproduces asexually at a fairly rapid rate. If a single specimen is placed in a depression slide containing culture fluid, and covered with a coverglass, the actual rate of reproduction can be determined. Several new individuals will be formed within a day or two.

22. Sensitivity of the Earthworm. The earthworm is much more sensitive to mechanical stimuli at its anterior or posterior ends than it is in the median portions. This may be shown by the use of a needle. The prostomium is especially sensitive to a variety of stimuli—tactile, photic, and chemical. The intact earthworm (*Lumbricus terrestris*) is negative to all except very weak light, to which it is positive. If the ventral nerve cord is cut, the portion of the worm anterior to the incision reacts negatively to light; that portion behind the incision reacts positively (Hess 1924). If the cerebral ganglia are removed, the entire worm reacts positively to light of moderate intensity. Sensitivity to light varies greatly in the various parts of the worm's body. Hess (1925) has shown that the degree of sensitivity varies with the number of photoreceptor cells present in the epidermis. Such cells are abundant in the prostomium, the first four segments, and the caudal segment. The segments near the middle of the body have very few. Photoreceptors are absent in most of the ventral areas.

23. Locomotion in the Earthworm. A comparison of the rate of locomotion on wet glass and on wet paper towelling shows the important role played by the setae in the process. The antagonistic action of the circular and longitudinal muscles of the body wall can be demonstrated by cutting a small transverse slit and a small lengthwise slit in the dorsal surface posterior to the clitellum (Welsh and Smith 1946). When the circular muscles contract the lengthwise slit gapes; when the longitudinal muscles contract, the transverse slit gapes.

24. Effects of Narcotics. A five per cent aqueous solution of urethane (ethyl carbamate) is useful as a gentle anesthetic. Earthworms placed in this solution become limp and insensitive in two or three minutes. Considerable mucus is secreted, but it does not coagulate. This reagent is excellent for demonstrating the circulation of the blood, and for any operations requiring complete recovery. The animal is restored to full activity by immersion in running water.

Chloroform vapor, on the other hand, causes extreme contraction of the circular muscles. It may be used to demonstrate autotomy. At random over the length of the worm, but more frequently posterior to the clitellum, the circular muscles of a segment may constrict to the point where the body is severed, or autotomized.

For preparing fully relaxed specimens for immediate dissection, or for preservation, a procedure using naphthalene in alcohol is recommended (Cole 1927, 1928).

25. Vascular Movements. For demonstrating the movements of the dorsal vessel and the "hearts," the specimen should be anesthetized lightly in urethane, the dorsal wall cut slightly to one side of the median line, and the body pinned out on a wax pad. The tissues should be kept moist with 0.6 per cent NaCl.

26. Demonstration of Nephrostomes. The

specimen is anesthetized in urethane. Then two or three drops of 1:2000 methylene blue should be injected into the body cavity at several points, until the worm is distended. After ten minutes the dorsal wall should be slit, the worm pinned out on a wax pad, and the digestive tract carefully removed. The nephrostomes, which have a special affinity for the stain, can be clearly seen *in situ* (Cole 1925, 1934b). Since a nephrostome projects through the anterior septum of the segment in which the bulk of the nephridium lies, it is difficult, in unstained specimens, to avoid tearing the nephrostome away from the rest of the nephridium. But, when the organ is stained by the method just described, there is little difficulty in making a complete dissection. The nephridium should be transferred to a slide and a coverglass applied. Features readily observable include the general form of the organ, the roundworms infesting it (Section 20), and the beating of the cilia covering the nephrostome.

(To be continued in February 1955 issue)

Postage Stamps and Biology Teaching

THOMAS R. COX
Student in Biological Education
Oregon State College

Teachers must of necessity be constantly on the lookout for new and better methods of presenting learning materials to their students. Many unique and original ideas evolve in answer to this constant challenge. Recently, I encountered a method which, I am sure, is not only unique, but also adaptable and effective. This method involved the use of postage stamps as a teaching device in biology classes.

When I first observed stamps being used as a teaching device it was done as an individual project in a high school biology class. The class was studying the different areas of the world, and how the plants and animals in them differed. It soon developed that most of the students were aware only of the usual run of foreign animals, elephants, tigers, polar bears, and the like; thus their thinking in regards to the fauna of any region was distinctly stereotyped. One boy in the class, however, collected foreign stamps as a hobby and soon adapted this avocation to a project which greatly aided the teacher in correcting some ideas of the class, as well as influencing several other students in the class to take up this educational hobby.

The project, as completed, consisted of several ten by twenty inch cards each representing some region of the world. Displayed on these cards were neatly arranged stamps from one or more countries in that particular region. The stamps selected were ones depicting the animals of a given country. Since many countries have issued very attractive series along this line, a wide and representative cross-section was available for each region. The beautiful colors and pictures, combined as they were with samples of the native language, made the display an immediate hit with the students.

In this first project seven regions were represented: Australia, The Latin American Highlands, The Latin American Tropics, The Cold Northlands, The Old World Deserts, India and the Far East, and Africa South of the Sahara. In the lower left hand corner of the card a small world map was reproduced, and the countries represented on that particular chart were colored. After the stamps and pertinent information had been placed on the card the whole thing was covered with a sheet of cellophane. This protective measure made it possible for the students to pick up and examine at close range the individual cards without damaging or losing the stamps.

The novelty of this presentation method caused an appreciable increase in interest over more conventional methods of teaching this subject matter and, apparently as a result, the students seemed to have less difficulty in altering their preconceived notions to fit the facts. Retention, too, proved high, and once again proved the maxim that interest and learning go hand in hand.

Like any project, the benefit was not all on the part of the class. The student presenting these cards found a way in which he, normally a rather bashful student, could contribute to the class. This, and similar projects which followed on other phases of the course, seemed to develop in him a feeling of self-confidence and I am sure he gained much

prestige in the eyes of his classmates as a result of his very different type of project.

Perhaps the thing which most strongly recommends this type project, however, is its versatility. It lends itself not only to presenting the animal life of an area but also the plants, agriculture, landforms, culture, economic life and a whole host of other topics.

Adapting a Camera Lucida for Drawing by Microprojection

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In a previous issue of this journal the authors presented a method of constructing an easily made microprojector for use on a vertical microscope for drawing purposes.[1] The device consisted of two front-surfaced mirrors in a mailing tube, utilizing the periscopic principle for bending light rays from a microscope horizontally and then downward onto drawing paper. For many types of drawings this method of tracing projected images was easier than using a camera lucida.

The authors have incorporated a camera lucida in a similar type of microprojector which is even easier to construct than the previously described one. For those who possess a camera lucida, the only other materials needed are one front-surfaced mirror and a cardboard tube.

A cardboard tube was selected which would slide down over the ocular and the draw tube of the microscope. The one illustrated was from a mailing tube which had a metal end crimped to the ends of the cardboard for extra strength. This tube was notched to hold the mirror at a 45° angle over the top of the ocular. The notch was made by inserting a wooden rod into the tube for support while sawing part way through both the tube and the rod at a 45° angle. With the wooden rod in place, a ¾ inch drill was used to cut a hole in the wall of the tube to provide an outlet for the light beam reflected from the center of the mirror (Fig. 1).

The lower part of the tube was notched in such a manner that it could be pushed down over the ocular to rest on the top of the clamp of the camera lucida with the prism and filter portion of the camera lucida turned back out of the way, as shown in Fig. 1. The mirror should be almost in contact with the knurled rim of the ocular. Care must be taken to prevent the mirror surface from being marred.

The inside of the tube was painted with waterproof black drawing ink to reduce reflections. Black lacquer was used on the outside. After the photograph was made, black plastic electrical tape was used to seal the notch holding the mirror to reduce the entry of dust.

As shown in Fig. 2, another temporary mount for a front-surfaced mirror can be made by cutting a 45° angle slot in a short piece of thick-walled rubber tubing. Cellulose tape, as shown in the illustration, or a rubber band can be used to hold the tubing against the draw tube of the microscope with the slot just above the top of the ocular.

A 45° angle microprojection prism could be used in lieu of the two types of mirror mounts mentioned above, but mounted prisms are expensive and most of these cannot be clamped to the draw tube of the microscope while the camera lucida is clamped in place. An unmounted, silvered prism, obtainable from war surplus supply companies, can be used if a soft ring of rubber or other material is utilized to support the prism above the ocular in such a way as to prevent damage to the upper lens.

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[1]Davis, L. R. and Bowman, G. W. An Easily Constructed Microprojector for Drawing Purposes, Amer. Biol. Teacher, Vol. 15 (6): 150-151, 1953.

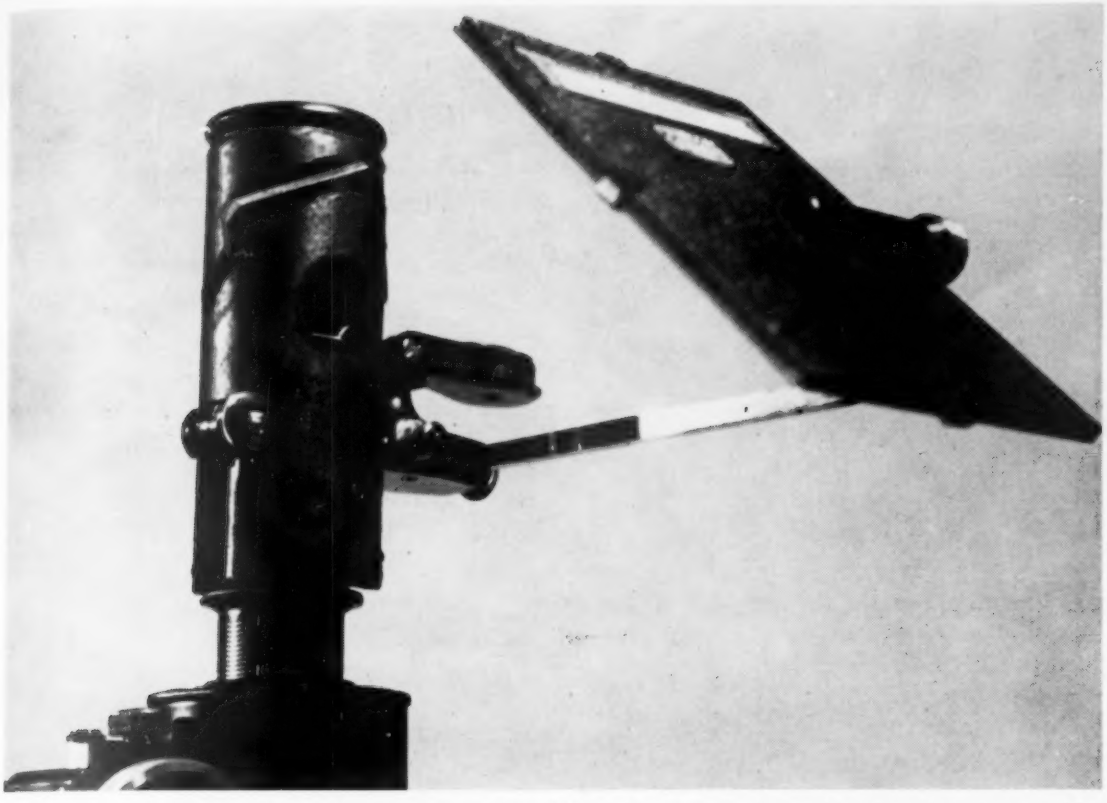

FIGURE 1. A front-surfaced mirror supported in a 45° angle slot over the ocular of a microscope enables a camera lucida to be used for projecting an image onto drawing paper for tracing.

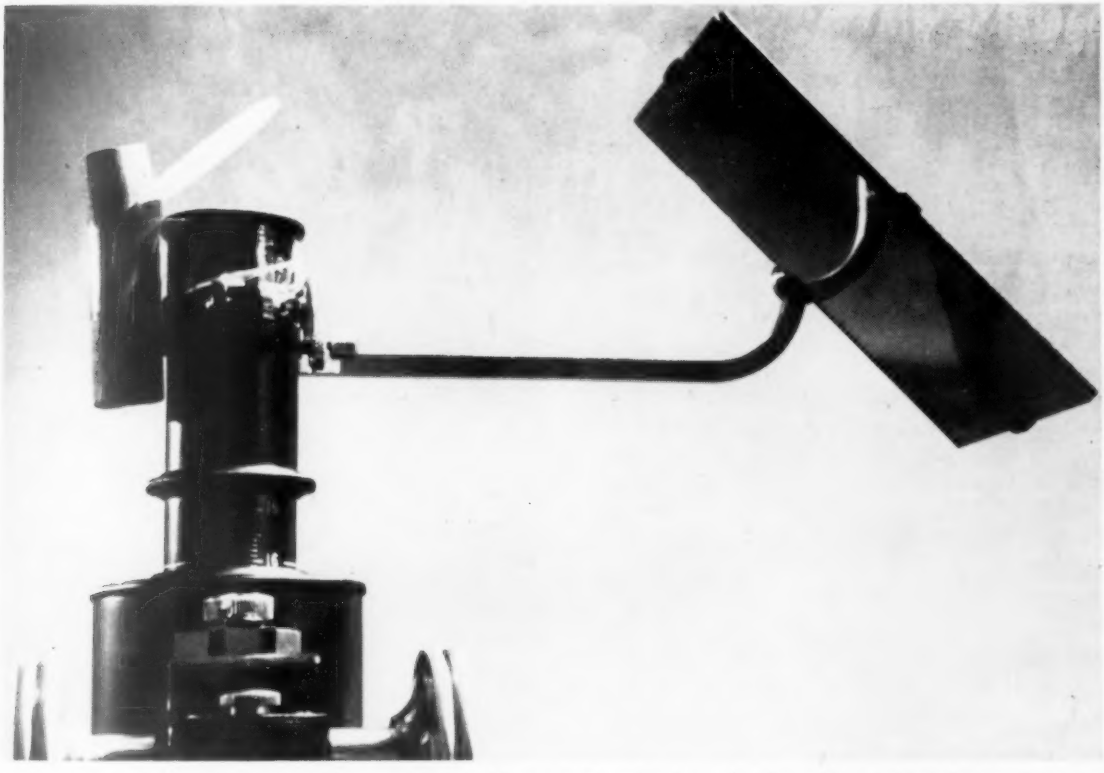

FIGURE 2. A front-surfaced mirror can be held at a 45° angle in a slot in rubber tubing fastened to the microscope with cellulose tape.

Plant Parasitic Nematodes

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Nematodes are a group of animals which, in the past, have generally been neglected in high school and even college biology courses. Nematodes, or eelworms, are representatives of a large group of animals which are commonly called the roundworms. Some are parasites causing such diseases as trichinosis and hookworm in man. Others attack our domesticated and wild animals. Many live in fresh or salt waters and enormous numbers of nematodes live in the soil either as saprogenous or parasitic animals.

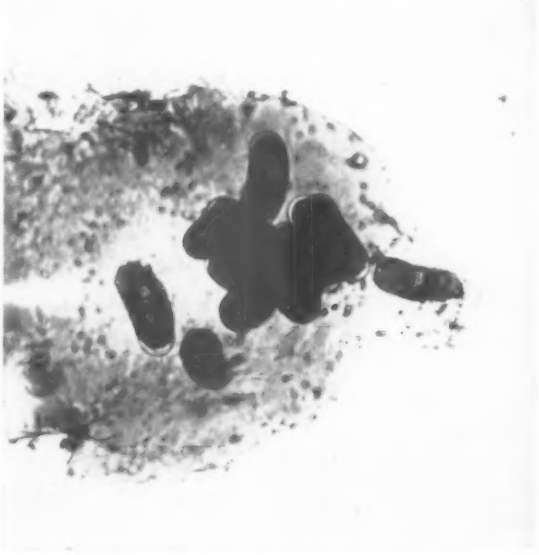
Probably the most important group in the soil are the root-knot nematodes. The root-knot nematodes are parasites living in the roots of plants and capable of distinguishing their hosts by a chemical sense organ. A simple laboratory exercise will be proposed which will demonstrate the activities of these soil nematodes in relation to their host plants.

The plant parasitic nematodes are long, slender animals, whose external surface is smooth and glistening, tapering to a point toward one or both ends. They occur in large numbers in all normal soils where plants are growing. In size they range from less than one sixty-fourth of an inch to approximately one-eighth of an inch in length while their diameter varies somewhat depending upon the particular stage in which they are found. Generally, the male is much smaller than the female. Because of their size they can easily be seen with the low power objective of the miscroscope or with a hand lens.

Their small bodies have specialized organs for feeding, a digestive, muscular, nervous, and excretory systems and a well developed reproductive system. There is no definite circulatory system, and respiratory organs are lacking. The muscular body wall makes it possible for the body to be knotted, curved or bent, and permits the characteristic undulatory movements.

Root knot nematodes.

Shortly after the primary infection is completed the nematodes become sexually mature, they pair and soon the female enlarges and eggs are produced. A mature female may lay as many as 300 to 600 eggs. When the roots are heavily parasitized the females frequently reproduce parthenogenetically.



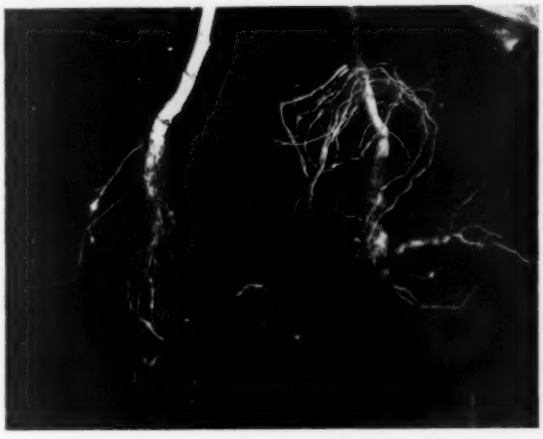
Nematode egg masses in a root knot.

The life cycle of the root knot nematode is relatively simple. After escaping from the egg mass in the soil the young larvae begin immediately to feed upon the roots of susceptible plants. Starting with this initial feeding stage, they pass through several developmental stages. Each larva bores into a young root by the use of its hollow buccal spear and with it sucks up some of the liberated cell contents. Usually the parenchymatous tissue is penetrated, which stimulates the host cells to undergo rapid reproduction. This reproduction results in enlargement and distortion of the invaded tissues. Such an enlargement of the root is commonly referred to as a knot or gall, and because this knot is a characteristic symptom, the nematodes forming it are called the root-knot nematodes. The knots may appear as slight swellings, as small, scattered, tubercle-like growths or as extensive swellings 1 or 2 inches in diameter on either large or small roots. In extreme cases it may involve nearly the complete root system. If the knots are cut across, from one to several dark specks may be noted in the cortex, marking the location of the nematode. In other cases, the organisms may produce an immediate yellowing of the plant which is frequently accompanied by stunting of the whole plant.

Seldom are the host's vascular and woody tissues invaded, although they may be twisted and misplaced through the pressure of the enlarged parenchymatous tissues.

All plant parasitic nematodes are somewhat specific, attacking some plants freely and others not at all. When a resistant host is present, the nematodes live saprogenously in the soil. Approximately one thousand plants are susceptible to nematodes. The algae, mosses and fungi are resistant to attack by nematodes. In fact, numerous species of fungi attack and destroy nematodes.

Early in the fall bring into the laboratory some light, sandy garden soil and place it in a pot. Then plant some seeds of a plant susceptible to infection and water them every other day. After the seedlings are above ground the general appearance of the plant, if the roots are infected, will give the impression that it is suffering from a lack of fertilizer and water, even though they are available in the soil in abundance. The color is a lighter or more yellowish green than normal.



Root knots on tomato plants, caused by nematode infection.

The choice of indicator plants to use to determine if the root-knot nematodes are present in the soil is limited only by one's imagination, but the two plants commonly employed for nematode infection studies are tomato and lettuce. These plants are used because it takes only about 14 days for germination of the seeds and approximately 25 days for the nematodes to complete their life cyle.

After the above symptoms are apparent, pull up the plants with their roots attached, and gently wash them under tap water. The knots or galls are then easily seen. Place a few knots in a watch glass with a few drops of water and tear them apart with needles, and then either place the watch glass under the lower power objective of the microscope or take some of the contents and place them on a slide and observe. What you see will be the complete life cycle of plant parasitic nematodes unfolding before your eyes.

Because of the specificity of nematodes, the soil which is brought into the laboratory may not contain nematodes capable of attacking the indicator plants. If this proves to be the case, some nematode infected plants can probably be obtained from a local greenhouse, as infestation of greenhouse soils and planting stock is very prevalent. An inspection of the roots of plants exhibiting typical symptoms should reveal root knots. Crush the knotted roots and place them in the soil.

Another interesting experiment would be to have students bring in plants from their family garden and check them for nematode infection.

The Use of Projects in Biology[1]

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Many of you may use projects in your biology teaching. My project method has evolved during a number of years of teaching, and it is my own since I have never had a course in the teaching of biology. Somebody passed around the word in educational circles that I have been somewhat successful in promoting projects in my classes so I have been asked to tell you about it.

My first experience with biology projects came when some of my pupils requested outside work in order to get extra credit, and to make better grades. One pair of students made a rather extensive collection of insects and brought it to class as a surprise. They were asked to make a report on the methods they used and I found that they did not even have an insect-killing bottle but managed to kill a number of insects by smothering them in a small vial.

This set me to thinking that perhaps most of the class members would like to do some individual work and that I should do a better job of directing it. After doing some extensive reading on the subject and thinking it over at length, I decided that some class time should be allotted to projects, and to use pupil-teacher planning. After a unit of work had been completed my pupils and I listed projects related to it on the chalkboard. At first I had to suggest most of the projects, but soon some of the lively members of the class were making suggestions. We then discussed the projects, how they were to be carried out and where to find help. A few were removed from the list when they were thought to be impractical. The pupils then chose their subjects and started to work, individually or in pairs, unless the nature of the project required a larger number of students to carry it out.

Some projects were very popular and the pupils had to draw straws for these. A few youngsters had no preference and were assigned a subject.

Before this took place, I had explained the nature of our work to the librarian and asked her to assemble materials for us. I also collected materials and had them in readiness. Some pupils quickly made their plans and went to work. Others wanted to discuss their plans with me before they started. Some merely wasted time and had to be pushed into getting started. I found it necessary to budget my time among students in the classroom, laboratory, and library, and be available at all times for those seeking help. I also went on field trips. The original deadline for the completion of the projects had to be extended because we had underestimated the time needed to complete a satisfactory job.

When the work was completed and project reports had been given, we found that many of the students had done a splendid piece of work and had enjoyed doing it; however, some had completed a rather dull and uninteresting piece of work, lacking in originality; and a few had failed to finish their work.

[1]Presented at a joint meeting of the Florida Association of Science Teachers and the National Association of Biology Teachers as part of the The American Institute of Biological Sciences program, Gainesville, Florida, September, 1954.

We discussed the reasons for the failures and tried to point out the ways of overcoming difficulties. The general opinion was that we had worked rather blindly, but that we had gained some valuable experience.

During the next unit of work students were constantly asking when we were going to work on other projects. When the time came for project activity connected with this unit, there were many more suggestions by the students. Most of them were anxious to get started. This time every member of the three sections of biology completed their projects. On the whole, much better work was done.

Since that time my pupils have worked on many projects, and I am constantly delighted and astonished at their accomplishments. Projects seem to be the best way to work with a heterogeneous group of boys and girls who show divergent interests and widely separated abilities. The advanced student can put extra hours of preparation into his work and he is not held back by the slower members of the class. Projects well done give the student a sense of personal accomplishment; some pupils who are rather poor at literary work can do a superior piece of work in collecting, building, or identifying. Those with artistic ability can find an outlet for this in biology. Projects are often the means of introducing new interests and new avenues of learning to students. One boy stated that he liked to do projects "because we make better grades on them than we do on other work."

The pitfalls of this method of teaching are many. Pupils who are accustomed to formal discipline by a strict teacher take advantage of the comparative freedom of project work. Many loafers find this an ideal time to continue loafing unless given some incentive to work. It is rather difficult to get pupils to budget their time.

What are some of the projects that high school boys and girls have done? One girl wanted to grow some unusual plants and ended by growing them in a terrarium, building six other terraria, and presenting her exhibit and a paper on them at the Florida Junior Academy of Science. Her paper took first place. Two girls who were interested in blood study asked for help at a hospital, and after they had obtained the information and help they needed, they were offered part-time jobs which they held for three years. Now both are studying nursing. A boy who decided to study and make a collection of mosses found to his amazement that there were four species in the school yard. Another boy made quite an extensive collection of club mosses. Two girls who worked with molds, unearthed two articles on slime molds, and had the class, teacher, and others interested in biology, searching for slime molds for the next few months. A boy made a comparative study of vertebrate brains. Two girls produced a sort of "peep show" depicting a Carboniferous landscape complete with club mosses, horsetails, fern trees, and dinosaurs. Quite a lot of research and originality went into this project. Four girls who had been "problem children" in the ninth grade built an insect marionette show to present to their class. They wrote the script, original words to familiar tunes, and rehearsed until they were quite deft at making caterpillars undulate across the stage, bees buzz around from flower to flower, and flies flit about. Their show was such a hit that they showed it to all the science classes in school, and finally took it to the Florida Junior Academy of Science, and won a prize. Their pride was as great as if their prize had been a fortune! There were no other marionette shows until the sister of one of the first group reached biology class, and that year six girls teamed together to give another marionette show—a review of the entire phyla of animals beginning with protozoa and ringing down the curtain with ape and man.

Many other successful projects could be mentioned. Skits, dioramas, collections, models, charts, unusual experiments, and an endless variety of projects attract youngsters. One idea often leads to another. Those of you who have never attempted project work with a group of lively youngsters have a delightful experience in store for you. One of the greatest thrills I have had as a teacher is to see these projects materialize and go to successful completion. When a pupil, who has been rather indifferent in class, does an outstanding piece of work, I feel that he is really interested, and I try to find some means of holding that interest. Enthusiasm is contagious and an enthusiastic teacher will stir up enthusiasm among her students.

Growing Fern Prothallia

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The following method has been successfully employed in growing fern prothallia for classroom and research use. Spores from a number of genera have been utilized; however, spores from various species of *Osmunda* have been the ones ordinarily used.

Fertile fern leaves or leaflets bearing almost mature sporangia are placed on clean pieces of smooth paper. As the sporangia dry the spores are discharged onto the paper. After a quantity of spores has accumulated on the paper, they are put into number 1 gelatin capsules for storage until the spores are to be planted.

The gelatin capsules containing the spores are placed in small bottles which are then tightly stoppered. At average summer room temperatures the stored spores of *Osmunda* retain their viability for about three months, in a 40° F. refrigerator they retain their viability for about a year and a half, and in a 5° F. refrigerator viability has been retained at a rather high level for more than thirty months.

A mineral nutrient agar made with a modified Beijerinck's solution is used for the germination of the spores and for growing the prothallia. The formula for the stock solution of this mineral nutrient is as follows:

Ammonium nitrate (NH_4NO_3)	5 grams
Magnesium sulfate ($MgSO_4$)	2 grams
Monobasic potassium phosphate (KH_2PO_4)	2 grams
Calcium chloride ($CaCl_2$)	1 gram
Distilled water	1000 cc.

This stock solution is diluted in the proportion of 1 cc of stock solution to 9 cc of distilled water. One cc of a saturated solution of ferric chloride is added to a liter of the diluted stock solution just before it is used. The same nutrient solution is used for the germination of moss spores and the growth of moss protonema, but for mosses it has been found that a 1:99 dilution of the stock solution is more satisfactory than the 1:99 dilution used for ferns.

Agar is added to the diluted stock solution in the amount of 16 grams per liter. This mineral nutrient agar is sterilized in an autoclave at 15 pounds pressure for thirty minutes. A kitchen pressure cooker may be used if an autoclave is not available. After sterilization the agar is allowed to cool slightly and then is poured into sterile petri dishes to a depth of approximately one quarter of an inch. When the agar has cooled and jelled the spores are planted.

When the spores are to be planted, the contents of one of the gelatin capsules is emptied into a prescription bottle or flask containing 100 cc of sterile distilled water. After thoroughly agitating the spore suspension by blowing into it through a pipette, 1 cc of the suspension is taken out and spread over the surface of the agar in a petri dish. The lid is replaced and the dish is tilted back and forth a few times in order to obtain an even distribution of the spores. If the spores are fresh and viable this concentration will give a large number of germinating spores—far too many for good growth of the prothallia. Therefore, it is advisable to dilute this spore suspension. This dilution is made by taking 1 cc of the spore suspension and adding it to another 100 cc of sterile distilled water. This new suspension is agitated thoroughly and liquid is drawn up and blown out of the pipette several times in order to wash all of the spores out of the pipette into the water. One-cc portions of this second suspension are placed in the petri dishes containing the agar, and the dishes are tilted back and forth to spread the suspension evenly over the agar. To get still fewer prothallia per petri dish, another dilution may be made. A 1:25 dilution of the second suspension is suggested.

After planting of the spores, the petri dishes are illuminated by either natural light from windows with a north exposure or artificial light from fluorescent lights. The fluorescent

lights used by the author were regulated by a clock and the period of illumination extended from 5 a. m. to 10 p. m. This period of illumination was decided on in an arbitrary manner and not from any particular evidence except that it had been observed that cultures of *Euglena* sharing the illumination grew better when there was less than 24 hours of continuous illumination.

After about two weeks, petri dishes containing the 1-cc portions of the original spore suspension will become green and various stages in development of young prothallia will be evident. After about six weeks, prothallia producing sex organs will be found in the petri dishes which were planted with 1-cc portions of the diluted spore suspensions. If not crowded the prothallia will show the form characteristics of the species planted. Ordinarily sporophytes will not develop unless the cultures are flooded with water to provide a medium for the movement of the antherozoids to the egg. If the fern species utilized as a spore source is one in which apogamy occurs, sporophytes develop without flooding.

Occasionally contamination of the cultures by fungi may occur. Most fungi grow poorly on the mineral nutrient agar and thus do not interfere with the growth of prothallia. Mites and small insects have at times tracked in the spores of fungi which have contaminated the cultures.

Prothallia may be left in the petri dishes for several weeks. However, after two to three months the agar will dry down somewhat and crack. Prothallia may be transplanted to fresh agar if it is desired to maintain them for a longer time. Some old prothallia proliferate freely, and new cultures of prothallia may be started by picking off the adventive prothallia and placing them on fresh agar plates.

The Mid-west Conservation Education Conference was held at Higgins Lake Conservation Training Camp at Roscommon, Michigan, October 10-13, 1954. Responsibility for the program was shared by Dr. G. W. Mouser, Michigan State College, and Dr. R. L. Weaver, the University of Michigan.

Biology in the News

BROTHER H. CHARLES, F.S.C.
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SHOULD YOUR BOY PLAY FOOTBALL?, Al Stump, *American Mag.*, October 1954, pp. 26, 109-113.

A sports writer gives some shocking reasons for the mounting toll of accidents on high school gridirons. The safety code which is included is worth considering by students and administrators alike.

THE RAIN FOREST, Lincoln Barnett, *Life*, Sept. 20, 1954, pp. 76-106.

The ninth in the series "The World We Live In." The color pictures and the descriptions of life in a tropical rain forest are equal to those which have preceded it in the series. Several copies of this article should find a place in your file of really worthwhile bulletin board materials.

THIS SCHOOL IS READY FOR THE H-BOMB, Herbert and Dixie Yahraes, *Sat. Ev. Post*, Sept. 25, 1954, pp. 45, 111-114.

Do you have disaster training in your school? Would you be prepared to meet emergencies if your city were bombed? The plan described in this article might be used, with a few modifications, in case of other types of disasters. This article might excite lively discussion about what protective training your school should have.

BEFORE YOU DIET, Ethel Strattan, *Cosmopolitan*, October 1954, pp. 46-49.

Thumb nail discussions of twenty key theories on dieting. A thoughtful reading will provoke many reactions from students.

12 DIETS FOR YOU AND YOUR FAMILY, Herbert Pollack, M.D., *Woman's Home Companion*, October 1954, pp. 109-112.

This article, based on recent research, contains good news for those who would diet successfully to gain weight, to lose weight or to maintain normal health.

THE GREAT CARNATION LOTTERY, Frank J. Taylor, *Sat. Ev. Post*, Oct. 9, 1954, pp. 28, 112-115.

Denver's nearly 300 days of sunshine each year has enabled its carnation growers to develop new varieties and to take top honors in kinds and carnation production in the United States.

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. Books for Biologists .

WILD FLOWERS AND HOW TO GROW THEM. Edwin F. Steffek, Associate Editor of *Popular Gardening Magazine*. 192 pp. $3.95. Crown Publishers, Inc., New York 16, New York. 1954.

A practical book on the cultivation of wild flowers, with full information on where to find them, how to transplant them and raise them in the home garden.

Soils, seasons, water, light, drainage, climate, plant enemies, nutrition, etc. are discussed. The illustrations, in full-color and black-and-white are carefully keyed to descriptions: statements of where the flowers grow, their blooming seasons, species, and cultures.

WITHIN THE LIVING PLANT. Erston V. Miller, Professor of Botany, University of Pittsburgh, formerly Physiologist, United States Department of Agriculture. 325 pp. $12.00. The Blakiston Company, Inc., New York 22, New York. 1953.

This textbook for an advanced, undergraduate plant physiology course has two major objectives: (1) it presents fundamental principles of plant physiology; (2) it then focuses attention on the manner in which plant physiology may affect the everyday life of the individual.

ZOOLOGY. Clarence J. Goodnight, Associate Professor of Zoology, Purdue University, and Marie L. Goodnight, formerly Instructor in Biology, Purdue University. 700 pp. $6.50. The C. V. Mosby Company, St. Louis 3, Missouri.

A new type zoology text to provide the necessary technical background for students in the curricula in general agriculture, preveterinary, predental, premed, prepharmacy, medical technician and zoology, as well as the humanistic appeal that should be an integral part of biology.

After a brief introduction to the study of science, scientific method, protoplasm, classification, and other background material, the book emphasizes vertebrate study. Following the chapters on vertebrate anatomy and physiology, the book returns to material on the cell which logically leads into embryology, genetics, and evolution. Next there is a survey of the animal kingdom, which presents type forms and stresses their biological and economic importance. Concluding chapters take up discussion of ecology and its many implications.

MODERN EXPERIMENTS IN TELEPATHY. S. G. Soal, Senior Lecturer in Mathematics, Queen Mary College, University of London; and F. Bateman, M. Sc. (Lond.). 425 pp. $5.00. Yale University Press, New Haven, Connecticut. 1954.

The subject of this book is a question of great importance to contemporary science and philosophy. If the evidence that some events in the external world can be perceived in a manner independent of ordinary channels of communication withstands critical examination, and if it appears that the extrasensory process has quite peculiar properties, particularly in relation to time, many mechanistic explanations that have proved so fruitful in modern science will require reconstruction. The authors of this book have considered all the available evidence and present the case in favor of telepathy that appears to some competent critics irrefutable. They describe their own experiments, emphasizing the extreme precautions taken to exclude sensory cues, self-deception, and statistical artifacts. Because of the extreme care taken in its preparation, this book may well raise serious doubts in the minds of many who hitherto have refused to examine its subject matter.

Professor G. Evelyn Hutchinson of Yale University contributes an introduction.

ELEMENTS OF ECOLOGY. George L. Clarke, Associate Professor of Zoology, Harvard University. 534 pp. $7.50. John Wiley and Sons, Inc., New York 16, New York. 1954.

Stressing the unity of the science, the book deals with the ecological interrelations of both plants and animals, and with the aquatic as well as the terrestrial environment. The principal factors of the physical environment, such as water, temperature and light, are treated individually before their combined action is discussed.

Similarly, the ecological relations of individual plants and animals are regarded as one step in the understanding of communities and populations. Lucid descriptions of natural situations are given to help develop the general principals. Complex terminology is avoided. About 200 illustrations, including graphs, diagrams, and photographs augment the discussion.

One new chemical compound "completely retards" the growth of a common strain of bacteria in the test tube, declared Donald F. Loncrini, a chemist engaged in graduate studies at the Florida State University. The active compound is trifluoro norvaline, one of a series of altered amino acids which were produced in a search for nonpoisonous bacterial agents, said Mr. Loncrini.

Just as a key fits into a lock, the "fake" amino acid fits into the germ cell at a spot where a vital amino acid would normally go, explained Mr. Loncrini, but because the substitute "key" is not a vital amino acid, the germ is deprived of nourishment and dies.